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Clinical and Biochemical Spectrum of Patients With RSH/Smith–Lemli–Opitz Syndrome and Abnormal Cholesterol Metabolism

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RSH/Smith–Lemli–Opitz (RSH/SLO) syndrome is an autosomal recessive malformation syndrome recently shown to be associated with a severe deficiency of cholesterol biosynthesis and markedly elevated plasma and tissue levels of 7-dehydrocholesterol (7-DHC), the immediate precursor of cholesterol in the Kandutsch–Russell biosynthetic pathway. Because these biochemical abnormalities permit a reassessment of RSH/SLO on biochemical criteria rather than less specific physical criteria, we review here the clinical and biochemical characteristics of our first 80 patients with abnormally increased levels of 7-DHC. The study population included 68 index patients and 12 additional relatives identified by quantification of 7-DHC and cholesterol in plasma, amniotic fluid, or cultured fibroblasts, lymphoblasts, or amniocytes. As demonstrated in other clinical syndromes when redefined biochemically, we have found a wider range of clinical expression of RSH/SLO than previously recognized. These newly recognized atypical RSH/SLO patients included several with no malformations other than syndactyly of the toes and, at the other extreme, patients with frank holoprosencephaly or multiple visceral anomalies who died in utero. Syndactyly of toes 2 and 3 was the most common malformation, occurring in all but one of 80 patients. The best biochemical predictor of clinical severity was the plasma cholesterol level, which decreased with increasing clinical severity. However, at least 10% of patients, including one newborn infant, had normal cholesterol levels at the time of diagnosis and would have been missed without specific quantification of 7-DHC. Not unexpectedly, several patients carrying a clinical diagnosis of RSH/SLO were found to have normal levels of all plasma sterols and apparently normal cholesterol biosynthesis in cultured cells. A comparison of the frequency of anomalies in our biochemically identified patients with similar data from previously reported clinical series suggests that up to 25% of reports of RSH/SLO in the literature may describe genetic conditions other than RSH/SLO with 7-DHC-emia. Am. J. Med. Genet. 68: 263–269, 1997.

KEY WORDS: RSH/Smith–Lemli–Opitz syndrome; 7-dehydrocholesterol; cholesterol metabolism; autosomal recessive inheritance; inborn error of metabolism

INTRODUCTION

RSH/Smith–Lemli–Opitz syndrome (RSH/SLO; McKusick #270400) has been known for more than 30 years as an autosomal recessive, multiple congenital anomaly syndrome comprising cleft palate, cataracts, ptosis, hypospadias, postaxial polydactyly, and a distinctive craniofacial appearance [Smith et al., 1964]. Although the pathogenesis of the syndrome was obscure for many years, the recent discovery of hypocholesterolemia and high plasma and tissue levels of 7-dehydrocholesterol (7-DHC) in patients with RSH/SLO suggests that RSH/SLO is caused by a fundamental disorder of cholesterol metabolism at the level of 3β-hydroxysteroid-Δ^7-reductase [Irons et al., 1993; Tint et al., 1994]. Since the initial reports describing 5 patients with RSH/SLO and markedly increased plasma levels of 7-DHC, the same biochemical abnormality has been found in most patients with a clinical diagnosis of

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RSH/SLO [Kelley, 1995; Tint et al., 1995], both those with the classical syndrome first described in 1964 [Smith et al., 1964] and those with the more severe "type II" phenotype delineated in 1987 [Curry et al., 1987]. The discovery of abnormal cholesterol biosynthesis in RSH/SLO has provided clinicians with a valuable test for confirmation of the clinical diagnosis of RSH/SLO and with new tools for determining the pathogenetic mechanisms underlying the prenatal and postnatal abnormalities of growth and development in RSH/SLO.

In our laboratory, we have found abnormally increased plasma or tissue levels of 7-DHC in 80 patients ranging in age from 16 weeks of gestation to 27 years. Although many of these patients had a firm clinical diagnosis of RSH/SLO before testing, others were ascertained by laboratory abnormalities, such as hypocholesterolemia or abnormal urinary bile acid profiles, or because of mental retardation in association with just one of the lesser malformations characteristic of RSH/SLO. The identification of this large number of clinically diverse patients with abnormal 7-DHC metabolism enables us to ask important questions about the clinical syndrome associated with this biochemical phenotype. We describe here the biochemical characteristics of RSH/SLO, the correlation between clinical phenotype and biochemical severity, and the implications of these findings with regard to RSH/SLO and other malformation syndromes.

METHODS

A total of 80 patients (68 index cases and 12 family members) with increased levels of 7-DHC in plasma or other tissues were identified by quantitative sterol analysis [Kelley, 1995]. The index cases tested included 55 with a clinical diagnosis of RSH/SLO, 2 with an abnormal urinary bile acid profile [Natowicz and Evans, 1994], 2 who had equivocally abnormal levels of very-long-chain fatty acids, 2 in whom the diagnosis of RSH/SLO was first suggested by the finding of a low plasma cholesterol level, 2 tested because of ambiguous genitalia disclosed by fetal sonography, 1 with polydactyly and holoprosencephaly, and 6 with unexplained mental retardation and isolated 2/3 toe syndactyly or apparently nonsyndromical cleft palate. Most samples were submitted to our clinical diagnostic laboratory to confirm an existing or tentative diagnosis of RSH/SLO or to evaluate an ambiguous clinical phenotype.

Plasma samples were collected without regard to the state of fasting and kept frozen on dry ice or at −20°C until analysis. Other sources of primary diagnostic material included skin fibroblasts in 3, frozen liver tissue in 2, amniotic fluid in 3, and cultured lymphoblasts, cultured amniocytes, and a newborn screening blood card in one patient each. Cholesterol and 7-DHC in plasma, amniotic fluid, cultured cells, and stored tissues were quantified by selected ion-monitoring gas chromatography–mass spectrometry as described previously [Kelley, 1995]. In most atypical RSH/SLO patients with 7-DHC-emia and in patients with a clinical diagnosis of RSH/SLO but normal plasma sterol profiles, the plasma or amniotic fluid results were confirmed in cultured cells or autopsy material. Karyotypic information was available on all 80 patients, 71 from peripheral lymphocyte culture and 9 from cultured amniocytes or skin fibroblasts.

A previously published clinical scoring system for RSH/SLO [Bialer et al., 1987] was used to assign a clinical severity score for the 75 patients on whom sufficient clinical data were available. In this scoring system, malformations of 5 major viscera (heart, kidney, gastrointestinal tract, liver, and lung), 3 lesser anomalies (cataracts, polydactyly, and cleft palate), mental retardation, and age at death are scored to give a value from 0 to 19. The separate severity score for genital malformations [Bialer et al., 1987] was not calculated because of incomplete description of the genitalia in many of the 46,XY patients. Clinical information for scoring was available from either personal examinations by one of the authors (17 patients) or detailed reports from the patients' physicians (58 patients).

RESULTS

Clinical Data

The group of 80 patients with abnormal plasma or tissue levels of 7-DHC in the index case or a similarly affected relative ranged in age from 16 weeks gestation to 27 years. Of 55 index cases referred for testing because of an established clinical diagnosis of RSH/SLO, only 18 (33%) were genetically female (46,XX), reflecting the disproportionate ascertainment of RSH/SLO by male hypogenitalism found in other patient series [Johnson, 1975; Jeanty et al., 1977]. However, a 46,XX karyotype was found in 10 of 22 patients identified by criteria other than a clinical diagnosis of RSH/SLO or ambiguous genitalia, a sex ratio closer to the 1:1 ratio expected for an autosomal recessive disorder. As in other genetic malformation syndromes, patients had a wide variety of combinations of malformations. Although no single malformation was present in all 80 patients, 79 of the 80 had abnormal syndactyly of toes 2 and 3. Several patients had a single discrete malformation characteristic of RSH/SLO, such as cataracts, minimal hypospadias, or 2/3 syndactyly, but had phenotypes that were considered nondiagnostic by the referring clinicians. Two patients with relatively severe forms of RSH/SLO had the unusual complication of progressive cholestatic liver disease, which was the cause of death in both. As expected, mental retardation of varying degrees was found in all patients for whom intellectual assessment was possible.

Table I compares the incidence of specific malformations in our biochemically defined RSH/SLO patients with the incidence in other series of clinically identified patients. Because the 2 earlier series were collected before the description or wide recognition of the more severe RSH/SLO phenotypes first delineated in 1987 [Curry et al., 1987], the malformation frequencies are listed for the complete series of 80 patients and for 61 patients with severity scores of 5 or below, which excludes all patients with RSH/SLO classifiable as type II, as well as a few patients with otherwise classical RSH/SLO. The higher frequencies of malformations in our biochemically identified individuals with RSH/SLO suggest that the older published series of RSH/SLO patients included two or more genetic disorders.

TABLE I. Smith–Lemli–Opitz Syndrome—Percent Anomalies

Malformation	Johnson [1975] $N = 55$	Bialer et al. [1987] $N = 121$	This series ($N = 77$) All	This series ($N = 77$) Severity < 6
Heart	"Occasional"	NR[a]	38	31
Renal cysts/ agenesis	NR	NR	13	0
Bowel	NR	NR	25	10
Postaxial polydactyly	22	26	43	34
Cataract	"Occasional"	10	18	13
Cleft palate	40	34	52	44
2–3 toe syndactyly	73	NR	99	100

[a] NR, not recorded.

Of the 80 patients in our series, 15 were ascertained for reasons other than an accepted clinical diagnosis of RSH/SLO in the index case or a relative. Although small, this group of patients is interesting because it indicates the future broadening of the RSH/SLO phenotype when ascertainment is by biochemical screening of developmentally abnormal children or by newborn screening [Zimmerman et al., 1997] rather than by clinical phenotype. Of these patients, 6 index cases and 2 sibs were tested because of developmental delays and the presence of a single discrete malformation, such as 2/3 toe syndactyly, but a phenotype otherwise considered nondiagnostic by the clinician. In one of these 6 families, a diagnosis of Noonan syndrome had been made in the father and the index case. Two patients identified because of significant hypocholesterolemia were severely affected and died of multiple visceral malformations, but had no external malformations other than 2/3 toe syndactyly. The indication for testing in another 4 patients was a suspected diagnosis of a peroxisomal disorder, such as Zellweger syndrome or neonatal adrenoleukodystrophy, which share some characteristics with RSH/SLO (hypotonia, cerebral dysgenesis, agenesis of the corpus callosum, and blepharoptosis). Two of the 4 patients had urinary bile acid profiles that suggested the abnormal sterol chemistry of RSH/SLO because of the near absence of normal bile acid species and presence of several compounds that appeared to be bile acids that were monounsaturated relative to normal urinary bile acid species [Natowicz and Evans, 1994]. Two other patients had mildly increased but nondiagnostic levels of very long chain fatty acids in plasma. In both of these patients, reports of abnormalities uncharacteristic of a peroxisomal disorder, such as polydactyly or 2/3 syndactyly, prompted sterol analysis of the same plasma sample and the finding of hypocholesterolemia and 7-DHC-emia. One patient with unilobar holoprosencephaly and polydactyly [Muenke et al., 1994] was identified by screening a library of lymphoblasts from patients with holoprosencephaly, a common malformation in animal models of RSH/SLO produced by pharmacochemical inhibition of 3β-hydroxysteroid-Δ^7-reductase [Roux and Aubry, 1966]. Of note, two other patients in our series had a midline cleft of the upper lip, a manifestation of the holoprosencephaly sequence.

Twelve patients were identified because of a previously affected sib or other relative with a clinical diagnosis of RSH/SLO and 7-DHC-emia. The 8 sib pairs (including both severe and mild phenotypes) were more alike than different in their severity scores, which when paired were 2–2, 2–3, 4–4, 17–13, 4–5, 2–2, 2–2, and 2–3. A similar correlation in severity of RSH/SLO within sibships has been noted in other series [Bialer et al., 1987; Curry et al., 1987]. No parents were known to be consanguineous, although 3 of 4 grandparents of one pair of mildly affected children were of American Cherokee heritage. However, in 4 of the 68 index families studied there was a second, nonconsanguineous sibship (of a first degree relative) with one or two biochemically proven or clinically unmistakable cases of RSH/SLO. These included 3 first cousins, 2 maternal aunts, and one paternal uncle. The relatively low incidence of consanguinity and high rate of secondary affected sibships in the case of an autosomal recessive disorder such as RSH/SLO support the hypothesis that RSH/SLO may have an incidence greater than 1:10,000 births and a carrier frequency of more than 1 in 50 [Holmes, 1994; Opitz et al., unpublished observations]. This apparent high frequency of heterozygosity for RSH/SLO may be restricted largely to Caucasians, because most patients in our series were of Northern European or Hispanic ancestry and patients of African or Asian heritage were absent.

Biochemical Studies

The fundamental biochemical abnormality in RSH/SLO appears to be a primary or secondary deficiency of 7-DHC-reductase (3β-hydroxysteroid-Δ^7-reductase) causing deficient synthesis of cholesterol and a corresponding marked increase in the level of its precursor, 7-DHC (Fig. 1). As expected, most patients with increased plasma levels of 7-DHC had abnormally reduced levels of cholesterol. The lowest value was 1.1 mg/dl (normal newborn level ± SD = 66.7 ± 10.3 mg/dl, $n = 10$) in a severely malformed newborn infant who died within days of birth of multiple internal anomalies. At the other extreme, the cholesterol level was greater than 100 mg/dl in 8 of 67 plasma specimens, with a maximum of 179 in a 3-year-old child with midline cleft lip, 2/3 toe syndactyly, and a plasma 7-DHC level of only 4.5 μg/ml. In some patients with very low

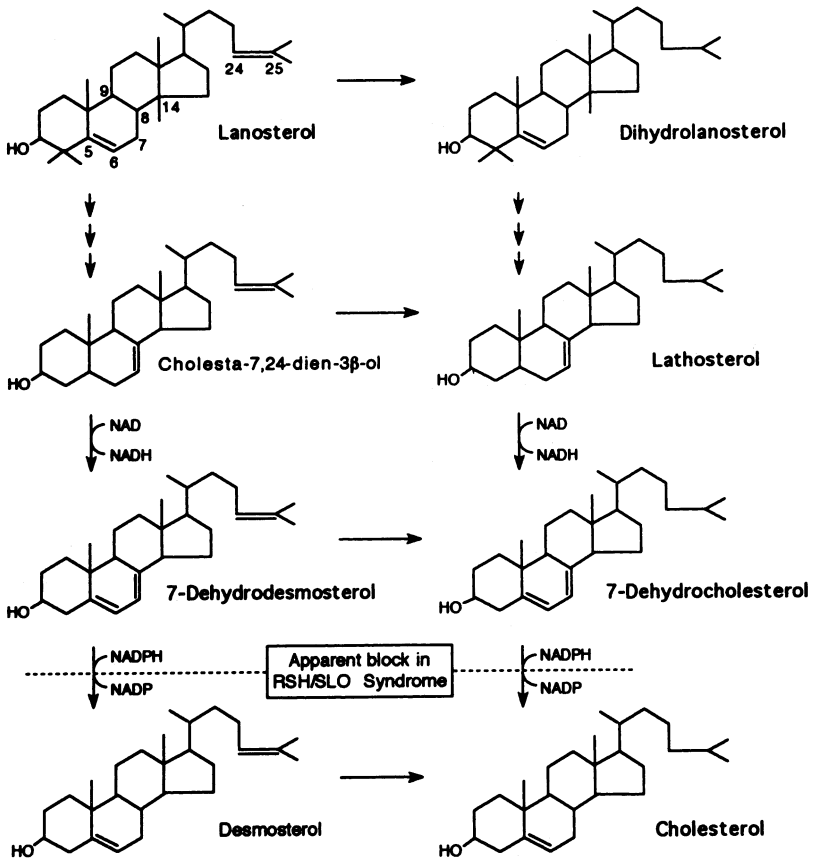

Fig. 1. Pathways of sterol metabolism from the first sterol, lanosterol, to cholesterol. The proposed defect in RSH/SLO syndrome is at the level of 3β-hydroxysteroid-Δ^7-reductase, which catalyses the conversion of 7-dehydrocholesterol to cholesterol and of 7-dehydrodesmosterol to desmosterol.

cholesterol levels by our GC/MS assay, normal cholesterol levels were reported by their hospital laboratories, most likely because 7-DHC cannot be distinguished from cholesterol by most nonchromatographic assay systems. Thus, as these cases illustrate, routine measurement of serum cholesterol cannot be relied on as a sensitive screening test for RSH/SLO.

Although the level of 7-DHC varied from a low of 1.7 μg/ml to a high of 470 μg/ml (normal ± SD = 0.10 ± 0.05 μg/ml, n = 52), the plasma diene sterol pattern was essentially the same in all patients, with a dominant peak of 7-DHC (cholesta-5,7-dien-3β-ol) coeluting with its epimer, isodehydrocholesterol, plus a slightly smaller diene sterol peak with chromatographic characteristics and a mass spectrum consistent with 8-dehydrocholesterol [cholesta-5,8(9)-dien-3β-ol] [Axelson, 1991; Batta et al., 1995]. In the plasma of some of the more severely affected patients and in amniotic fluid, there was also a mildly increased level of lathosterol (cholest-7-en-3β-ol), the immediate precursor of 7-DHC (Fig. 1) plus very small amounts of 2 as yet unidentified triene sterols.

Figure 2 shows a surprising difference in the correlation between the plasma levels of cholesterol and 7-DHC for infants vs. older patients. Whereas in patients less than age 2 years there is a suggestion of a weak positive correlation between the levels of cholesterol and 7-DHC, for patients 2 years and older there was a strong inverse correlation for the same measurements. Recent experience with cholesterol treatment of RSH/SLO suggests that dietary cholesterol may downregulate the synthesis of 7-DHC in surviving patients

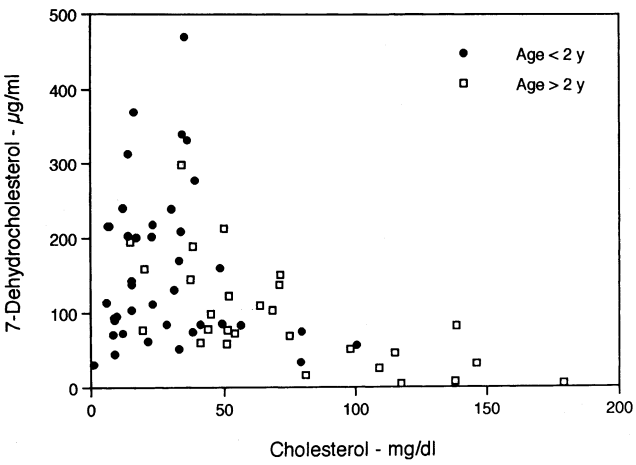

Fig. 2. Correlation of plasma levels of 7-dehydrocholesterol and cholesterol in patients with RSH/SLO syndrome.

with low severity scores. In most RSH/SLO children whose diets have been supplemented with from 50 to 100 mg/kg/day cholesterol from various sources, there was a steady decline in the level of 7-DHC from the time of diagnosis and a moderate increase in the level of cholesterol [Irons et al., 1994; R. Kelley, unpublished]. However, there also appears to be a genetic contribution to the variable sterol levels in that most affected sib pairs had relatively similar sterol levels regardless of age. For example, the newborn sib of a mildly affected 2-year-old RSH/SLO patient with plasma cholesterol and 7-DHC levels of 79.4 mg/dl and 75.4 μg/ml, respectively, had similar blood levels of cholesterol and 7-DHC of 56.3 mg/dl and 84.0 μg/ml.

To determine if there exists a correlation between clinical severity and the levels of cholesterol or 7-DHC, severity scores were calculated on patients with sufficient clinical data. Figure 3 shows that whereas there was little correlation between the plasma level of 7-DHC and clinical severity for children less than 2 years, there was a slight correlation between clinical severity and the level of 7-DHC for older patients. In contrast, there was a distinct inverse correlation between clinical severity and the plasma cholesterol level for all ages (Fig. 4). Some of the patients with the highest severity scores had increased but relatively low levels of 7-DHC (< 50 μg/ml) compared with most patients with classical RSH/SLO, whose plasma levels of 7-DHC are typically between 75 and 300 μg/ml. For example, one newborn infant with a severity score of 17 had a cholesterol level of 1.1 mg/dl and a 7-DHC level of only 30.9 μg/ml. In contrast, a much more mildly affected patient (score = 5) had a cholesterol level of 35 mg/dl and 7-DHC level of 470 μg/ml at diagnosis at 6 months [Nwokoro et al., 1994]. Tint et al. [1995] have also found that survival was lowest in the RSH/SLO patients with the lowest cholesterol levels. The better correlation of clinical severity with cholesterol levels than with 7-DHC levels suggests that the increased level of 7-DHC may not be as deleterious to the developing fetus as the low cholesterol level.

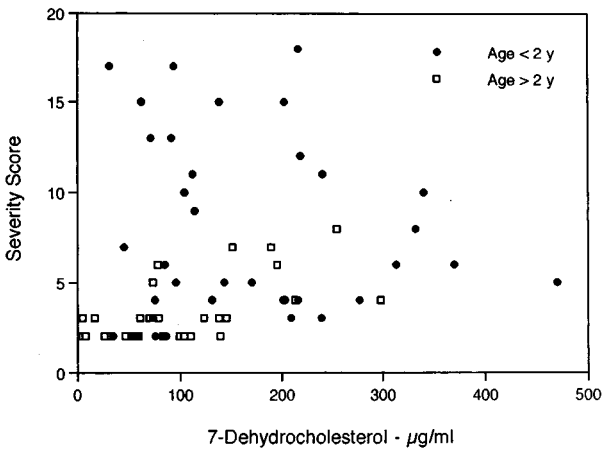

Fig. 3. Correlation of plasma 7-dehydrocholesterol levels with clinical severity scores of patients with RSH/SLO syndrome.

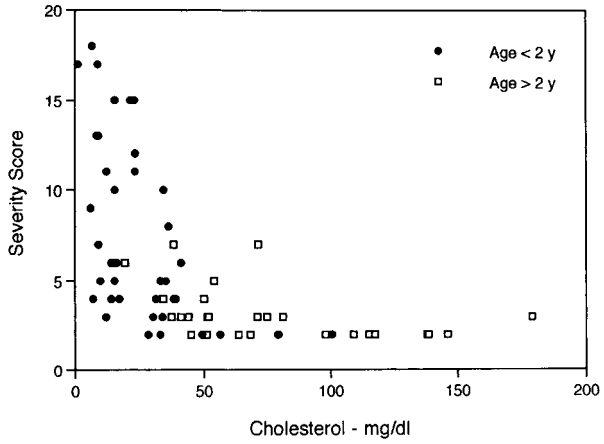

Fig. 4. Correlation of plasma cholesterol levels with clinical severity scores of patients with RSH/SLO syndrome.

A wide range of clinical severity in RSH/SLO has been recognized for many years. In their important description of "type II" RSH/SLO, Curry et al. [1987] considered the possibility that type II RSH/SLO may differ genetically from classical "type I" RSH/SLO. Although an early analysis of clinical severity in RSH/SLO found only a unimodal distribution of severity scores [Bialer et al., 1987], we found in our series a preponderance of severe and mild phenotypes over those of intermediate severity (Fig. 5A). However, when the severity scores

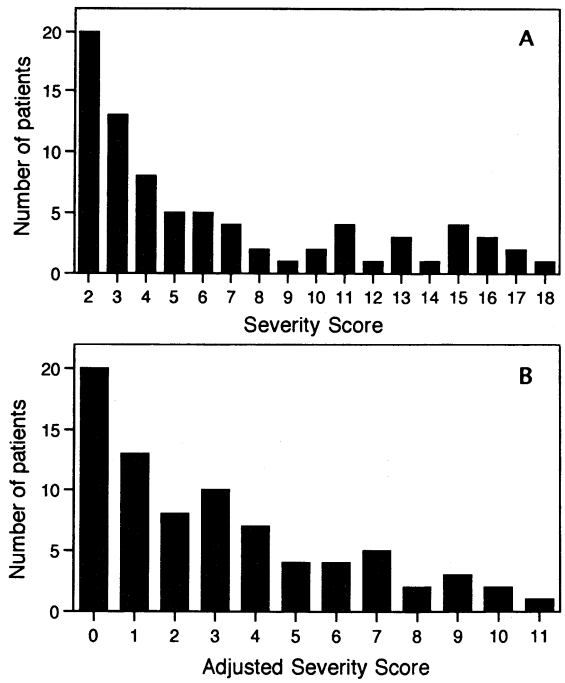

Fig. 5. Distribution of severity scores in 75 patients with RSH/SLO syndrome: (**A**) standard Bialer score; (**B**) Bialer score modified to exclude points for age at death and mental retardation.

are adjusted to eliminate the additional points for early death, which in effect "double weight" the internal anomalies largely responsible for death, a unimodal distribution of scores is evident (Fig. 5B). Nevertheless, the lack of clinical or biochemical evidence for genetically distinct type I and type II RSH/SLO does not preclude the possibility of multiple alleles causing the range in severity in RSH/SLO nor the existence of multiple genetic loci for RSH/SLO.

DISCUSSION

The discovery of an apparent primary defect of cholesterol biosynthesis in RSH/SLO, a syndrome previously known only as an autosomal recessive malformation syndrome, has important implications for the diagnosis and management of patients with RSH/SLO. In the same manner that the discovery of abnormal very-long-chain fatty acid metabolism in the cerebrohepatorenal (Zellweger) syndrome [Moser et al., 1984] initiated a revolution in the diagnosis and genetic understanding of peroxisomal diseases, the description of abnormal sterol metabolism in RSH/SLO will likely lead to an improved understanding of another class of genetic disorders. Indeed, RSH/SLO provides the best example to date of a much underemphasized concept in biochemical genetics, namely, that defects of metabolite biosynthesis can be the cause of genetic syndromes characterized principally by abnormal fetal development. The classical paradigm for an inborn error of metabolism is that of a infant with a defect of amino acid or organic acid metabolism who, when separated at birth from a compensating maternal/placental unit, develops a critical small metabolite deficiency or excess and suffers consequent neurological or somatic injury. In effect, the opposite paradigm obtains in RSH/SLO. As we have shown here, and as supported by animal studies of inhibitors of cholesterol biosynthesis [Barbu et al., 1988; Roux and Aubry, 1966], the inability to synthesize an essential nutrient, cholesterol, that is inadequately transported from the mother to the fetus [Carr and Simpson, 1982] leads to multiple abnormalities of morphogenesis. However, in the case of RSH/SLO, the deficient nutrient can be supplied in the diet after birth and, at least in some ways, benefit the patient. Anecdotally, those RSH/SLO infants who were provided from birth with a rich source of dietary cholesterol, such as breast milk, fared better and had fewer problems with growth retardation and recurrent infections. Moreover, in our preliminary experience with treatment of RSH/SLO patients with cholesterol, we have observed striking improvement in the growth and behavior of some RSH/SLO patients after beginning cholesterol supplementation (R. Kelley, unpublished observations). However, less certain is whether or not long-term treatment with cholesterol can improve cognitive development, especially in view of the frequent occurrence of congenital microcephaly and cerebral maldevelopment in RSH/SLO patients and in view of the evidence that minimal if any cholesterol crosses the blood–brain barrier [Morris and Chiakoff, 1961; Partridge and Mietus, 1980].

Another dividend of the study of cholesterol metabolism in RSH/SLO will be a better understanding of the genetics and clinical variability of RSH/SLO. As shown here, there is no justification at this time to consider type I and type II RSH/SLO to be separate genetic entities. Rather, there appears to be a continuum of clinical severity from mildly affected children with normal blood cholesterol levels at birth to lethally malformed fetuses and newborn infants with blood cholesterol levels less than 10 mg/dl. Nevertheless, the high concordance of clinical and biochemical abnormalities between affected sibs and the impression of some clinicians that there are clinically recognizable subgroups of RSH/SLO suggest that there exist multiple mutations of one or possibly several different genes causing RSH/SLO. Further study of the apparent high incidence of RSH/SLO and other aspects of its clinical variability and population genetics are now possible through biochemical screening of newborn infants. Guthrie card testing for RSH/SLO is simple and accurate by either standard gas chromatography/mass spectrometry [Kelley, 1995] or newer mass spectrometric techniques that can be more easily adapted to large volume newborn screening [Zimmerman et al., 1997].

It is important to note that not all patients with a prior clinical diagnosis of RSH/SLO who were tested through our laboratory were found to have abnormal levels of 7-DHC. However, of more than 500 patients tested because of a suspected diagnosis of RSH/SLO, fewer than 5 with normal plasma sterol levels were patients considered by experienced geneticists to have a clinical diagnosis of RSH/SLO. In several of these patients, we also documented normal sterol biosynthesis in fibroblasts or lymphoblasts [Kelley, 1995]. A number of older patients who carried the diagnosis of RSH/SLO, but in whom the appropriateness of the clinical diagnosis could not be assessed, were also found to have normal plasma levels of 7-DHC. However, because our standard method for plasma sterol analysis quantifies most known sterol intermediates between lanosterol and cholesterol [Kelley, 1995], it is unlikely that these patients with RSH/SLO-like syndromes and normal levels of cholesterol and 7-DHC have defects of cholesterol biosynthesis distal to lanosterol.

Although the reported affected sib frequency of only 18% for RSH/SLO [Johnson, 1975] could reflect a high rate of fetal death from heart defects or other malformations, another cause of a low sib ratio would be phenotypic overlap of RSH/SLO with one or more genetically unrelated, nonrecurrent malformation syndromes. In this respect, it is interesting to note that whereas we found 2/3 toe syndactyly to be almost universal among our patients with 7-DHC-emia, the RSH/SLO population described by Johnson [1975] had a frequency of 2/3 toe syndactyly of only 73%, suggesting that as many as ¼ of published cases of RSH/SLO may have a disorder genetically distinct from RSH/SLO defined as a defect of cholesterol metabolism. Our finding of apparently normal sterol metabolism in several patients with a clinical diagnosis of RSH/SLO underscores the importance of sterol testing in all patients with an established or suspected clinical diagnosis of RSH/SLO to provide appropriate genetic counseling to the families. With respect to patients with RSH/SLO

who died before sterol testing was possible and from whom pre or postmortem samples are not available, carrier status of relatives can be determined by analysis of sterol synthesis in cultured lymphoblasts (R. Kelley, manuscript in preparation) and may also be possible by DNA analysis in the near future [Alley et al., 1995].

The biosynthesis of cholesterol in humans is subject to complex regulatory controls, follows a long sequence of more than 20 reactions and transport processes occurring in microsomes, peroxisomes, and the plasma membrane. Yet, after mevalonic aciduria, RSH/SLO is only the second defect of cholesterol biosynthesis to be discovered. This dearth of knowledge about the human genetics of cholesterol biosynthesis contrasts sharply with our understanding of amino acid and organic acid catabolic pathways, for which almost all potential enzymatic deficiencies have been described as genetic disorders. We are clearly not looking in the right places or with the right tools for genetic defects of metabolite biosynthesis. However, our emerging understanding of the relationship between metabolite biosynthesis and congenital malformation syndromes exemplified by RSH/SLO will certainly aid in the completion of the human biochemical genetic map.

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